SUCCESS STORIES

Specific Genome Editing by TALEN and CRISPR-Cas9 technology Transcription activator-like effector nucleases (TALENs) are artificial restriction enzymes generated by fusing a TAL effector DNA binding domain to a DNA cleavage domain. TAL Nuclease has revolution the array of genome editing based on its ability to create a site-specific double-stranded DNA break (DSB). The break is then subsequently repaired by cellular machinery, through either homology-dependent repairs or non- homologous end joining (NHEJ), that creates a deletion mutation or a knock-in site for introducing site-specific DNA sequences of interest. Clustered, regularly interspaced, short palindromic repeat (CRISPR) RNA-guided nucleases (RGN) can robustly induce genome editing. Repair of RGN-induced double-stranded breaks by non-homologous end-joining or homology repair introduces insertion or deletion mutations or specific sequence alterations. The Streptococcus pyogenes Cas9 nuclease (Cas9) cleaves the intervening spacer sequence directed by a single guide RNA (gRNA) with a 20 nucleotides (nt) target complementarity region at its 5' end.

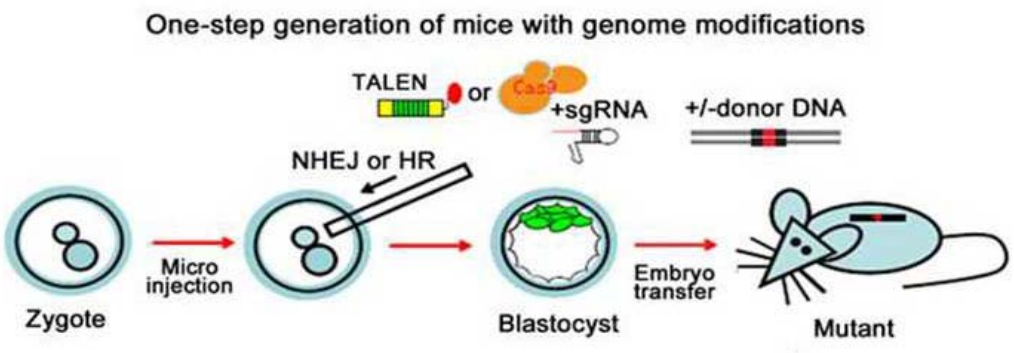
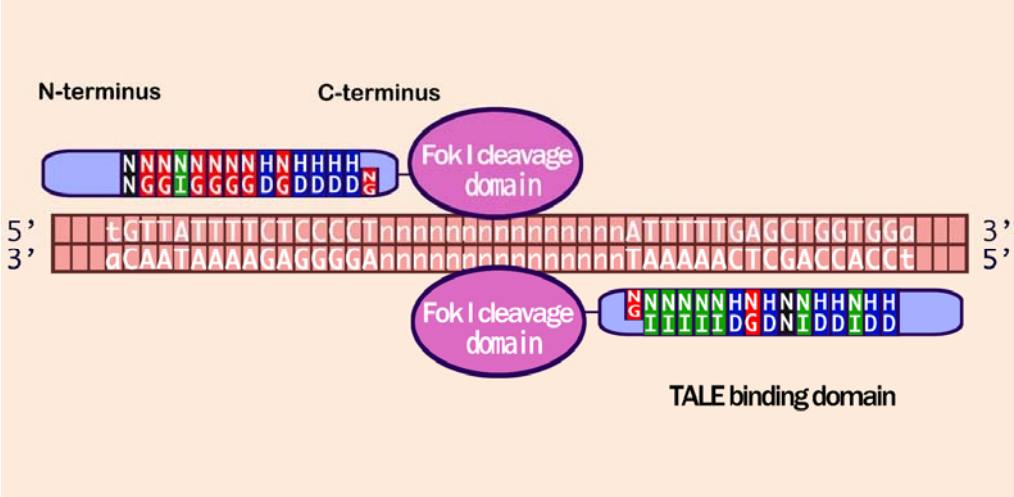


Table 1 describes the different strategies and technologies for gene targeting.

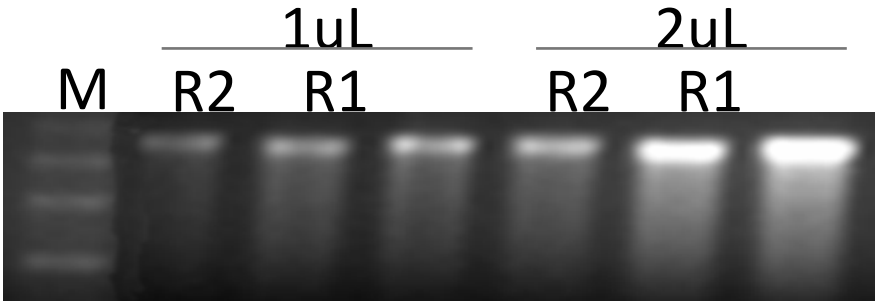
Table 1. Comparison of different gene targeting technologies

Property/Tools	ESCs-HR	ZFN	TALEN	CRISPR/Cas9
Type of recognition	HR	Protein-DNA	Protein-DNA	RNA-DNA
Methylation sensitive	sensitive	sensitive	sensitive	Not sensitive
Mutuation efficiency	Very low	high	high	Very high
Off-target effects	none	Less	Less	High
Multiplexing	Rarely used	Rarely used	Rarely used	Capable
Cost	High	Very high	High	Low

Story 1. Generating factor *FVIII* knockout animals with TALEN



TALEN expressed vector at 1 μL and 2 μL was examined at 1% agarose get, the intensity of RNA and its degradation were monitored prior to microinjection shown in the figure below.



We generated factor *FVIII* knockout mice by TALEN technology with the efficiency of 2.56-3.57% (Table 2).

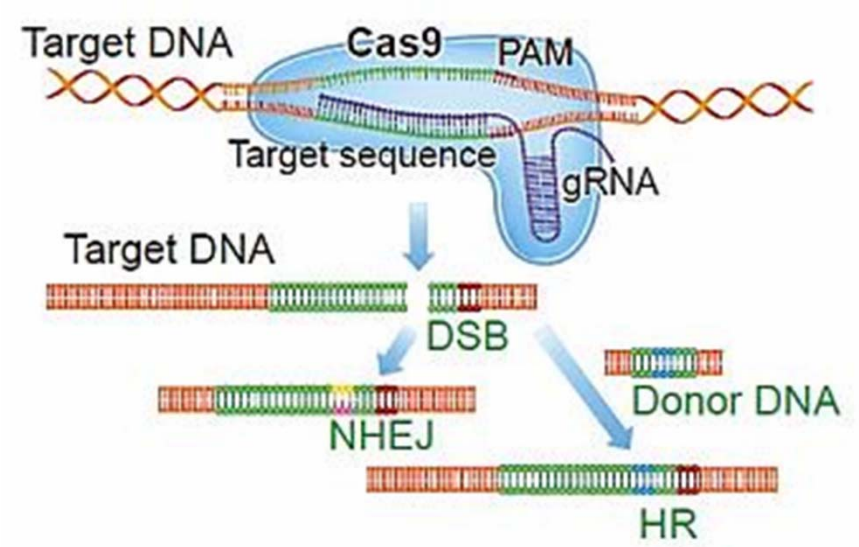
Table 2. Generating factor FVIII knockout mice with TALEN genomic editing

Talens pairs	Embryos injected	Newborn	Indels detection	Mutant mouse / embryos transferred (%)	Founder Name
L1+R2	96	0	0	0	
L1+R3	171	28	1（3.57%）	0.58	H32 ♂
L2+R1	120	20	0	0	
L2+R2	229	39	1（2.56%）	0.44	Q83 ♂
L2+R3	171	26	0	0	

Story 2. Generating factor *FVII* knockout animals with CRISPR-Cas9

Several strategies to improve specificity of the Cas9 system have been reported, such as the paired Cas9 nickase approach in which two gRNAs target adjacent sites on opposite DNA strands and each recruit a Cas9 nickase that nicks DNA instead of cutting both strands. This method can reduce off-target modifications at sites induced by single gRNA-guided Cas9.

Fu et al. reported that truncated gRNAs (tru-gRNAs) improved Cas9 nuclease specificity in U2OS.GFP and FT-HEK293 cells by shortening the gRNAs to 17/18 nt. They found that 5’-end nucleotides are not required for standard gRNA (std-gRNAs, 20 nt) activity and compensate for mismatches at unwanted positions along the gRNA target DNA interface, as shorter gRNAs are more sensitive to mismatches and therefore exhibit higher specificity. Here, we investigated the activity and specificity of tru-RGNs in inducing coagulation factor VII (FVII) gene mutations in murine cells and its efficiency in generating gene knockout (KO) mice by zygote RNA microinjection.



We generated factor *FVII* knockout mice by CRISPR-Cas9 technology with an efficiency as high as 78.9% (15/19) (Table 3).

Table 3 Generating *FVII* KO mice with gRNA and Cas9 mRNA co-injection

gRNAs	No. injected embryos	No. embryos transferred	No. recipients	No. newborns	Mutant alleles per mouse		Mutant mouse / total mouse tested (%)	Mutant mouse / embryos transferred (%)
					1(allve)	2(allve)		
F7-1	78	71	2	18	1 (1)	0(0)	1/18(5.6)	1.4
tF7-1	98	98	3	38	1(1)	18(0)	19/34(55.9)	19.4
F7-2	110	106	4	24	2(2)	6(0)	8/22 (36.4)	7.5
tF7-2	85	67	2	20	15(15)	0(0)	15/19(78.9)	22.4
F7-3	80	77	2	6	2(2)	0(0)	2/6 (33.3)	2.6
tF7-3	130	115	3	20	8(8)	0(0)	8/20(40)	6.9

Detection FIIV mutation frequencies by T7EI assay and subsequent sequencing (Table 4).

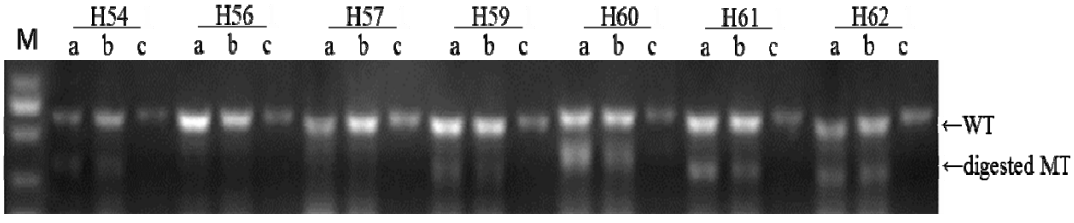


Table 4. Gene mutations in factor FVII knockout mice induced by CRISPR-Cas9

Founder	Target sequences (5'-3')	Indels
RGNs F7-1		
WT	AAAGGCGTGCCAACTCACTCCTGGAGGAGCTTTGGCCCGGCTCTCTGGAGAGAGA	
O68	AAAGGC------------------------TTTGGCCCGGCTCTCTGGAGAGAGA	-24 bp
RGNs tF7-1		
WT	CTGTTTCTCAGTTTTCATAACCCAGGAGGAAGCACATGGTGTCCTACACAGGCAAAGGCGTGCCAACTCACTCC	
I18	TTGTTTTTCAGTTTTCATAACCCAGGAGGAAGCACATGGTGTCCTACACAGGCAAAGGCGTGCCAACTCACTCC	C→T, C→T
RGNs F7-2		
WT	CCTACACAGGCAAAGGCGTGCCAACTCACTCCTGGAGGAGCTTTGGCCCGGCTCT	
I31	CTTACACAGGCAAAGGCGTGCCAACTCACTCCTGGAGGAGCTTTGGCCCGGCTCT	C→T
I34	CTTACACAGGCAAAGGCGGGCCAACTCACTCCTGGAGGAGCTTTGGCCCGGCTCT	C→T, T→G
RGNs tF7-2		
WT	AGGCGTGCCAACTCACTCCTGGAGGAGCTTTGGCCCGGCTCTCTGGAGAGAGAGTGCAAT	
H66	AGGC---CCGGCTCTCTGGAGAGAGAGTGCAAT	-30 bp
H67	AGGCGTGCCAACTCACTC---------------------------------TCTGGAGAGAGAGTGCAAT	-22 bp
H68	AGGCGTGCCAACTCA-----GAGGAGCTTTGGCCCGGCTCTCTGGAGAGAGTGCAAT	-6 bp
H69	AGGCGTGCCAACT------------------------TTGGCCCGGCTCTCTGGAGACAGTGCAAT	-16 bp
H71	AGGCTTG-------GCCCCT-------------------CC----TCTCTGGACAGAGTGCAAT	-25 bp
H72	AGGCGTGCCAACTC-------------------------------------TCTGGAGAGAGTGCAAT	-27 bp
H73	AGGCGTGCCAACTCACTCCTGAAG-AGCTTTGGCCCGGCTCTCTGGAGAGAGTGCAAT	-1 bp
H74	AGGCGTGCCAACTCACTCCTTGGAGGAGTTTTGGCCCGGCTCTCTGGAGAGAGTGCAAT	-6 bp
H75	AGGCGTGCCAACTCACTCC-----------------TTTGGACCGGTTCTCTGGATATAGTGCAAT	-16 bp
H76	AGGCGTGCCAACTCACTCACTTT∧GGAGCTTTGGCCCGGCTCTCTGGAGAGAGTGCAAT	+8 bp
	AATATGGA	
H78	AGGCGTGCCAACTCACT---------------TGGGAGAG----GG-TCTC------------AGTGCAAT	-27 bp
H79	AGGCGTGCAAACTC-----------------------TTTAGCTCTGCTC-------AC--AGTGCAAT	-29 bp
H82	AGGCGTGCCAACTCACCT-C------------AACTTTGGACCGGCTCTCTGGATATAGTGCAAT	-13 bp
H83	AGGCGT--	-167 bp
H84	AGGCGTGCCAACTCACT-----------------------------------CCGGTCTCAGGATAGAGAGTGCAAT	-27 bp
RGNs F7-3		
WT	CCTGGAGGAGCTTTGGCCCGGCTCTCTGGAGAGAGTGCAATGAGGAACACCTGG	
I66	CCTGGAGGA---AGTGCAATGAGGAACACCTGG	-24 bp
I68	CCTGGAG---AGAGAGTGCAATGAGGAACACCTGG	-24 bp
RGNs tF7-3		
WT	CCTGGAGGAGCTTTGGCCCGGCTCTCTGGAGAGAGAGTGCAATGAGGAACAGTGC	
H54	CCTGGAGGAGCTTTGGCCCGGCTCTCTGGAGAGAGAGTGCAATGACGAAGAGAGC	G→C, C→G, T→A
H59	CCTGGAGGAGCTTTGGCCCGGCTTCTCGGAAAAAAAAGGGAAAGGAGAAACATTGC	+1 bp
H60	CCTGGAGGAGCTTTGGCCTGGCTCTCTGGAGAGAGAGTGCAATGAGGAACAGTGC	C→T
H62	CCTGGAG--AGAGTGCAATGAGGAACTGTGC	-24 bp

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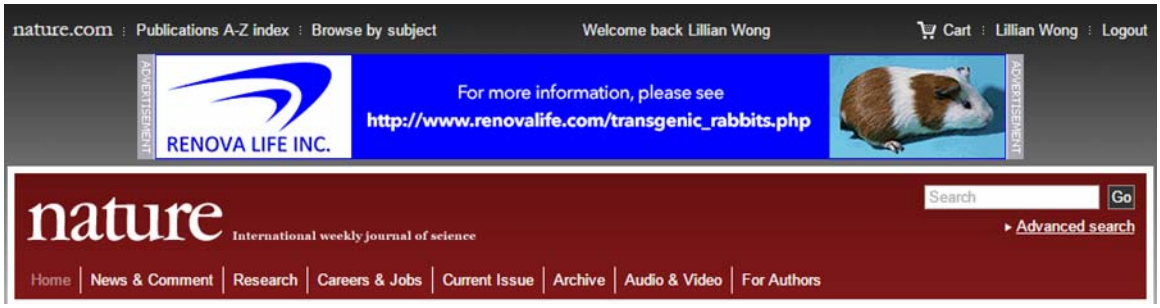

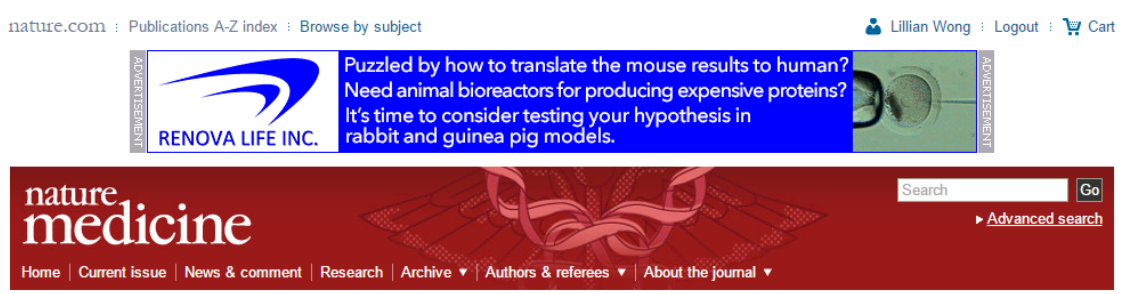

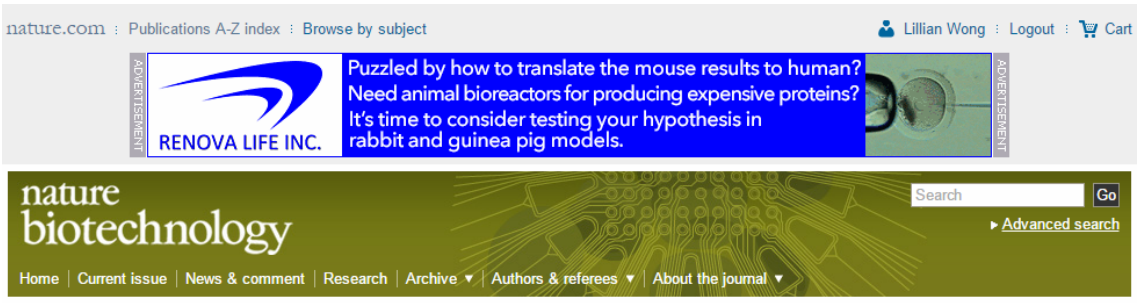

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